Exploring Transcriptional Silencing of Transposons in Zebrafish

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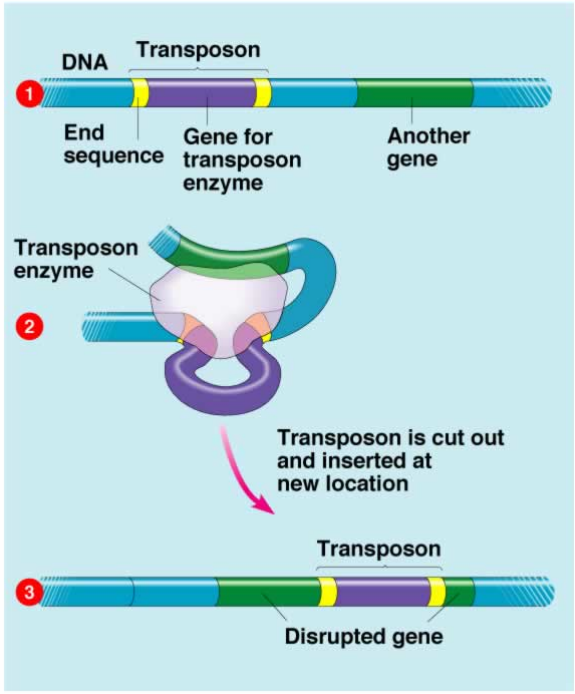
PURA Fall 2009 Project

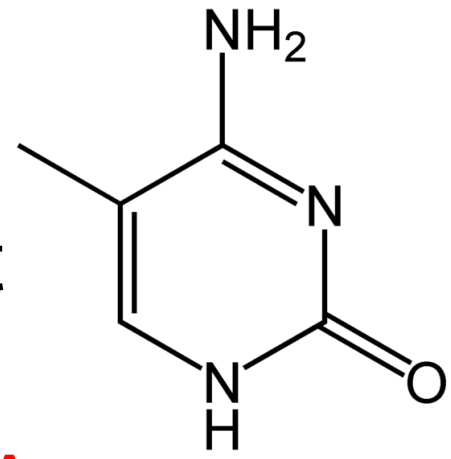
Transposons

- Parasitic DNA elements capable of invading host genome
- It is known that transposons are present in the zebrafish genome but it is unknown what role methylation plays in their silencing



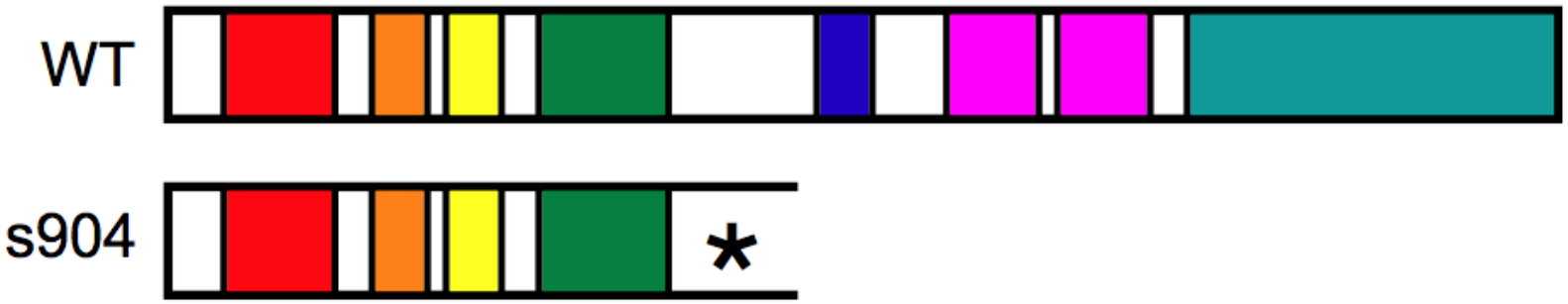
DNA methylation

- Epigenetic modification
- Can block the binding of transcription factors or recruit transcriptional repressors
- In plants and mammals, DNA methylation is essential for silencing of transposons



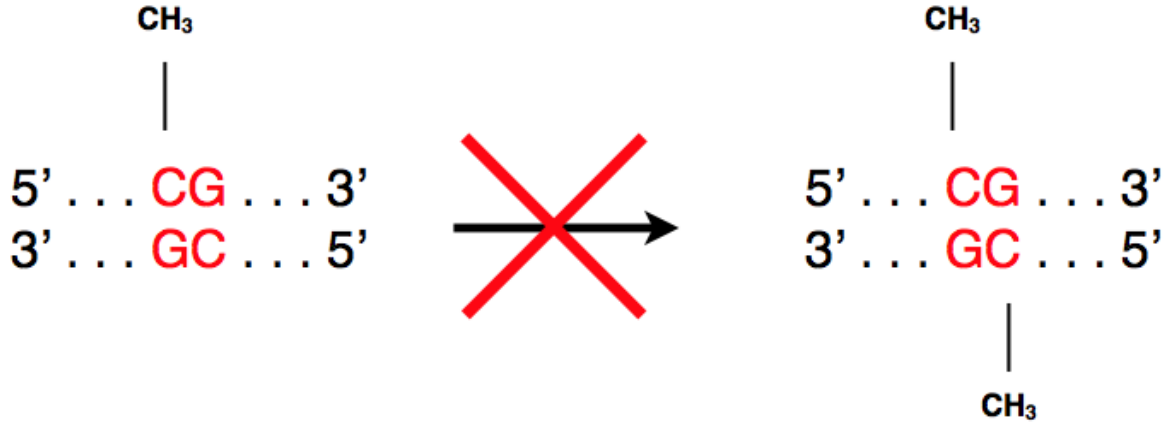
Does DNA methylation repress transcription of transposons in zebrafish?

Dnmt1 is responsible for maintenance of DNA methylation

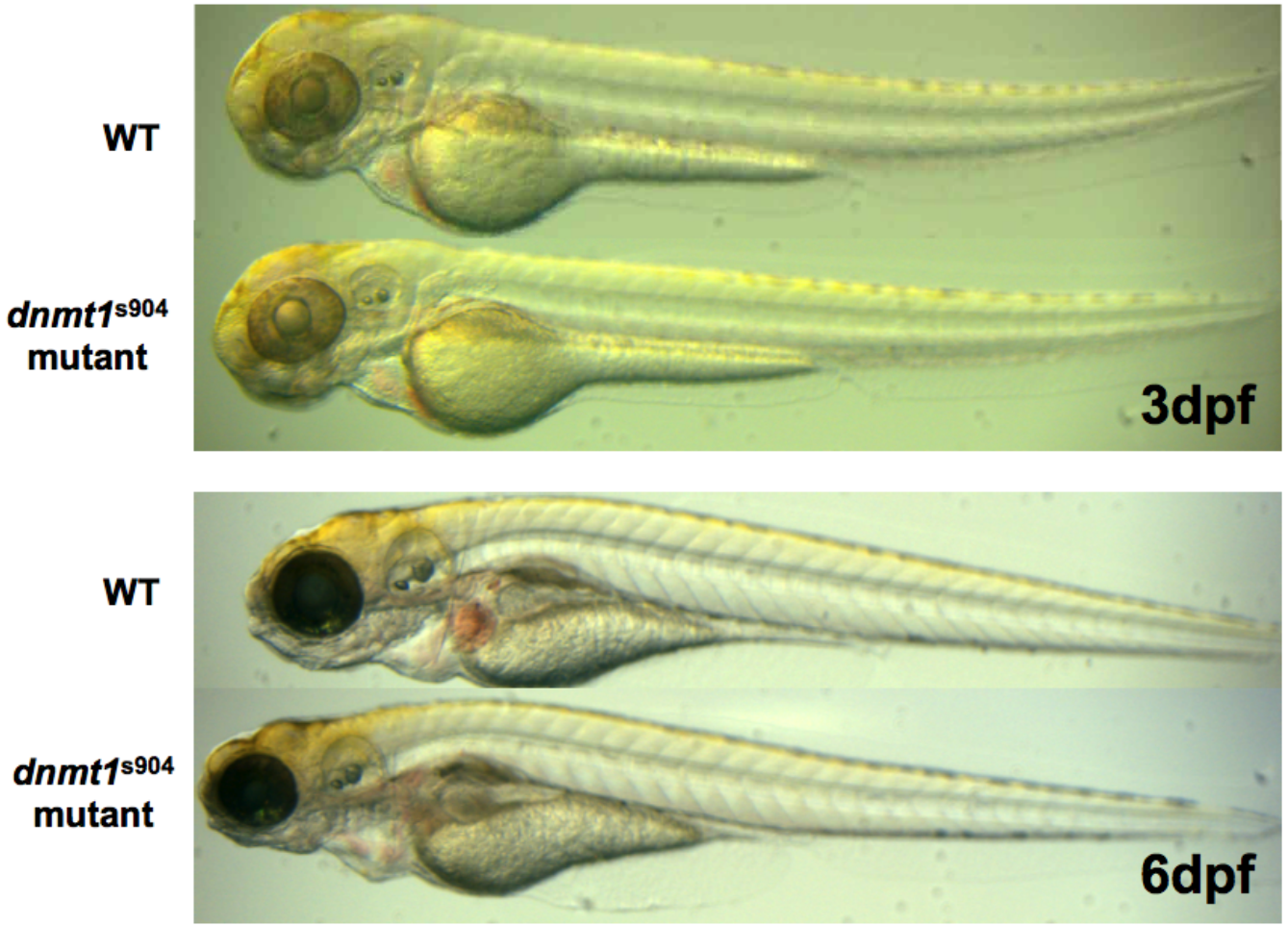


Anderson et al, 2009

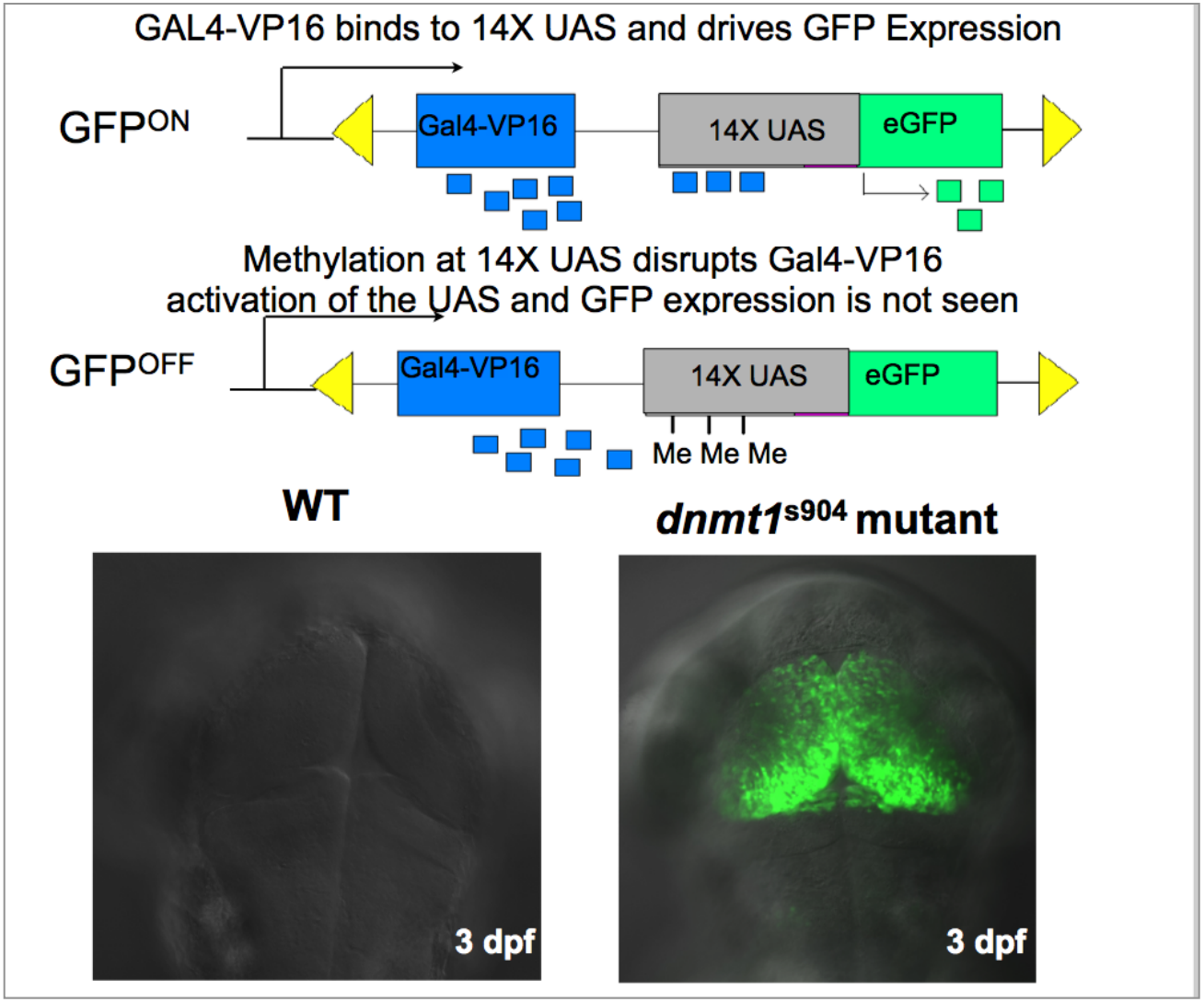
* s904 (*dnmt1* mutant allele) causes a premature stop mutation



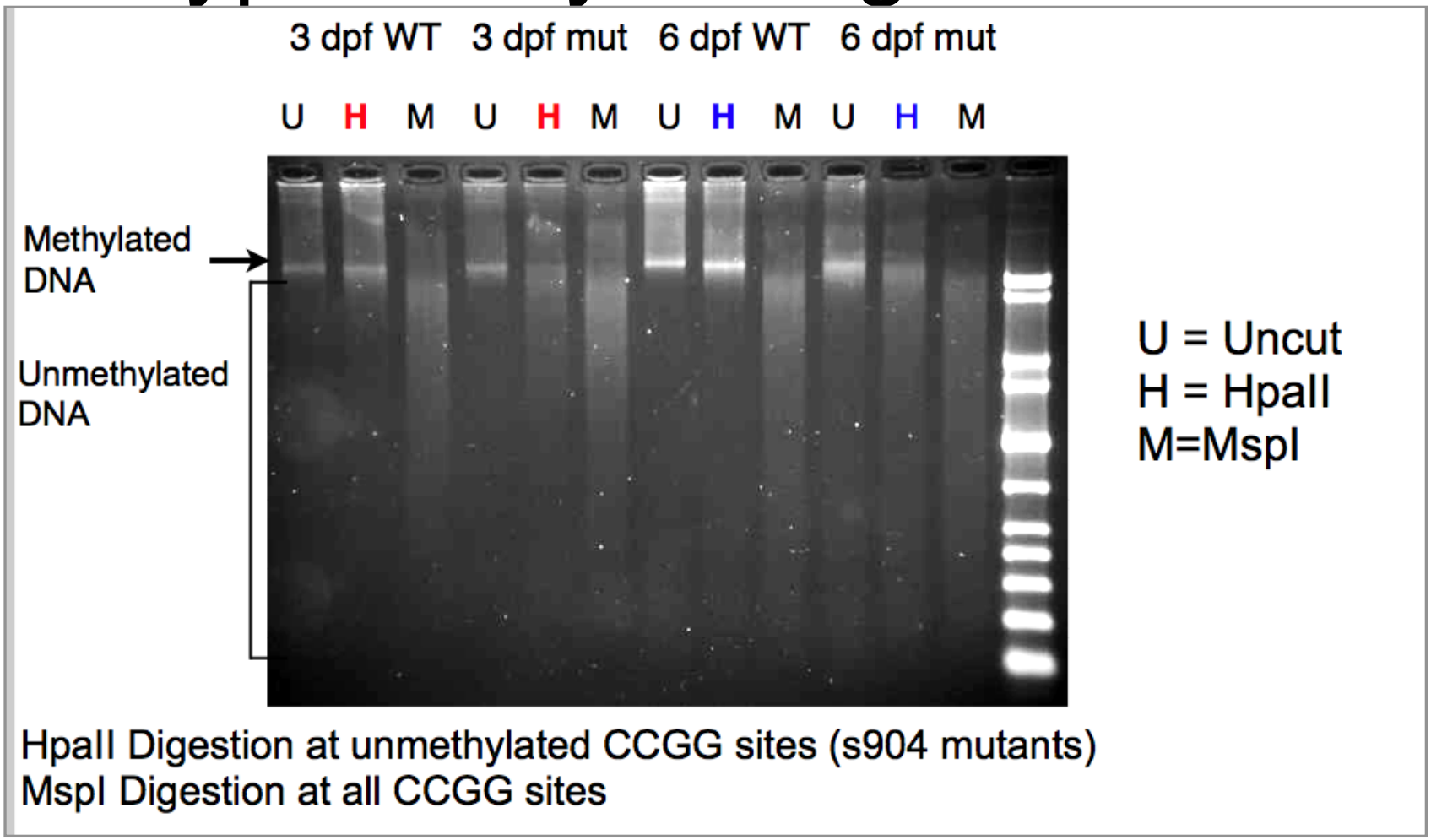
Dnmt1 mutants have many developmental abnormalities by 6 dpf



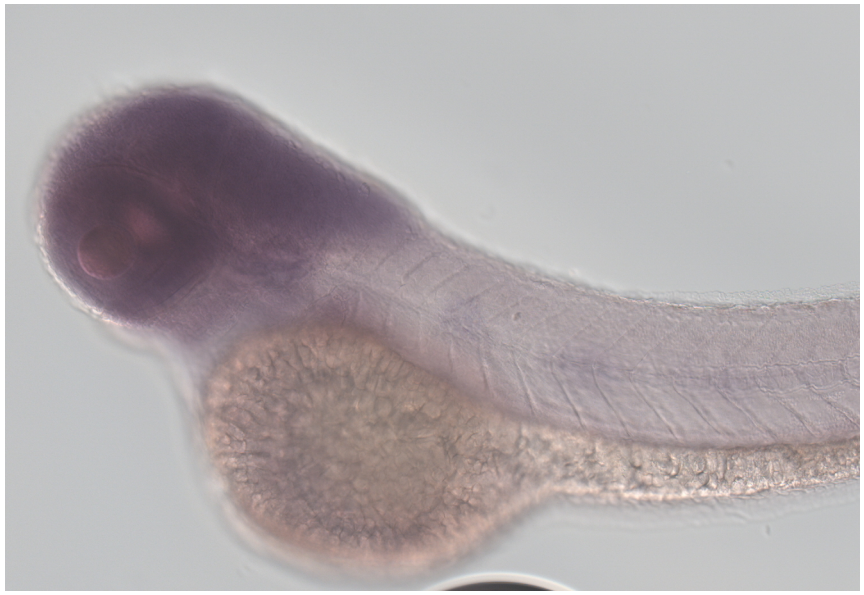
Visual assay to identify hypomethylated larvae

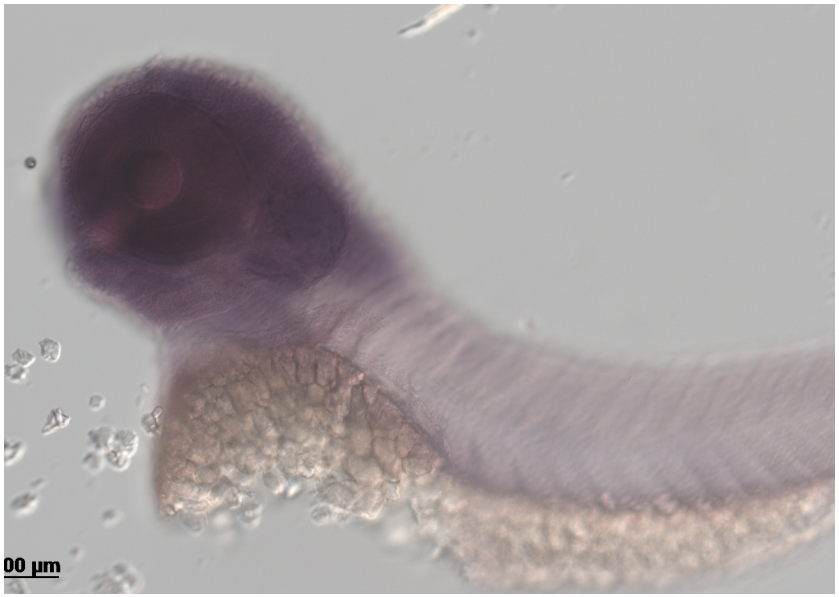


Dnmt1 zebrafish have hypomethylated genomes



In situ data using gypsy as a transposon probe





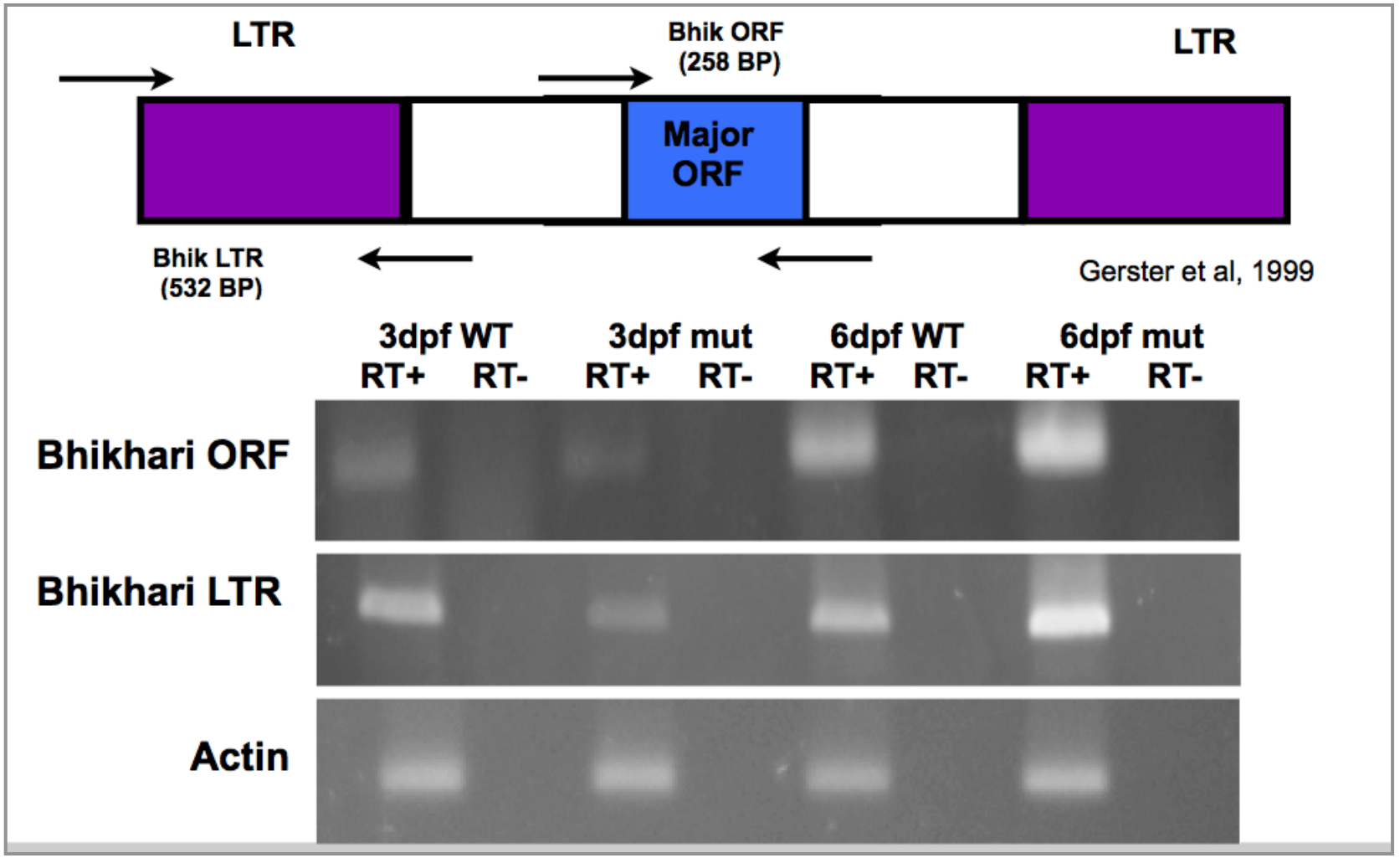
wt 3dpf ***dnmt1*^{s904} 3dpf**

In situs for ngaro, gypsy, line1-1, line1-3 were developed. These transposons are known to be controlled by the piRNA amplification loop in zebrafish (Houwing, 2008).

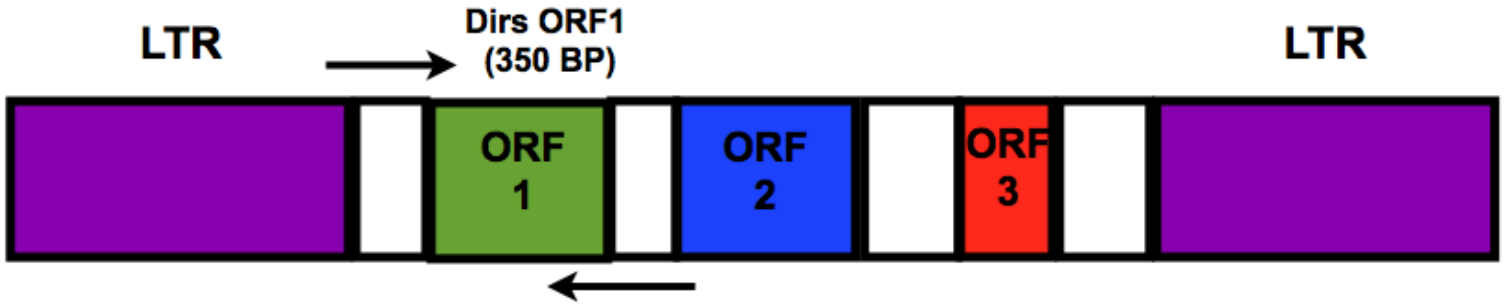
Examining Transposon Methylation and Expression in Zebrafish

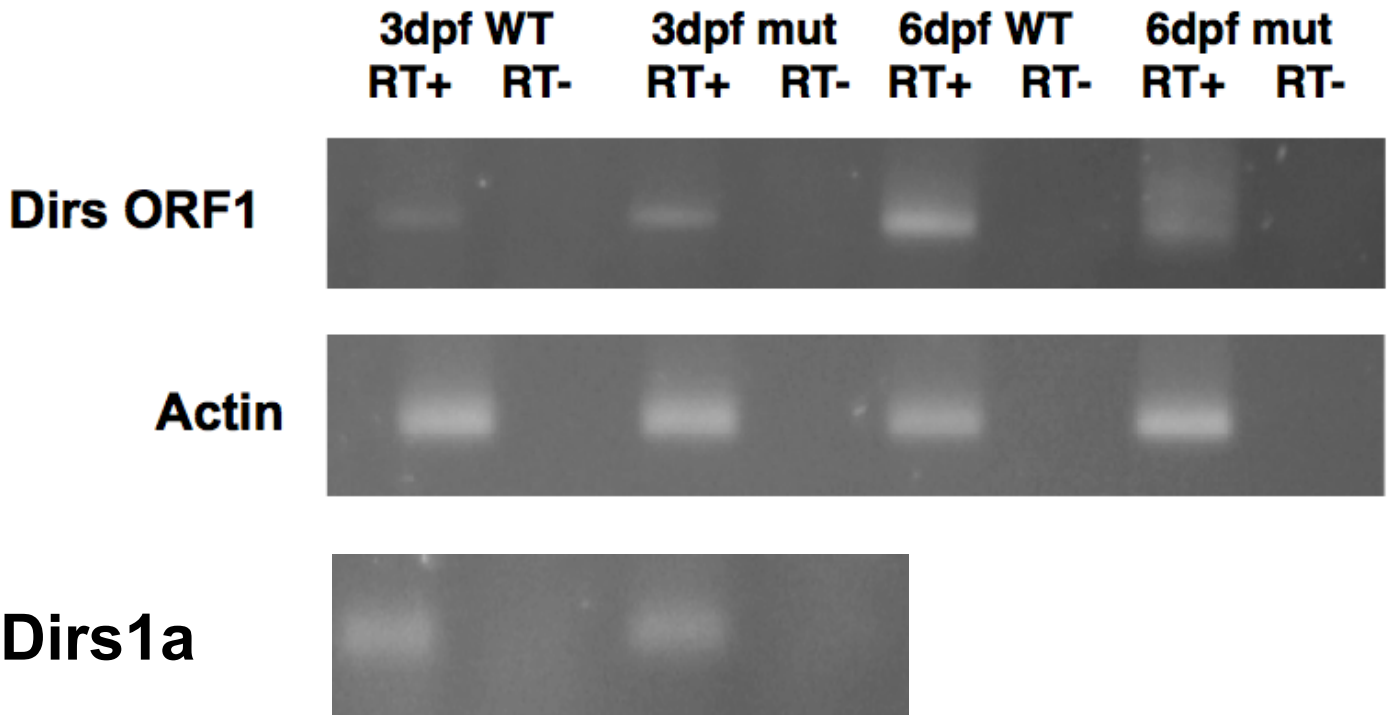
- Hundreds of transposon families in zebrafish genome
- Approximately 100-200 copies of Dirs and Bhikhari
- Dirs and Bhikari both have ORFS, suggesting they are active
- Used reverse transcription and PCR to amplify expressed sequences of Dirs and Bhik

Bhikhari


LTR
Bhik ORF
(258 BP)
LTR
Major
ORF
Bhik LTR
(532 BP)
Gerster et al, 1999
3dpf WT
RT+ RT-
3dpf mut
RT+ RT-
6dpf WT
RT+ RT-
6dpf mut
RT+ RT-
Bhikhari ORF
Bhikhari LTR
Actin

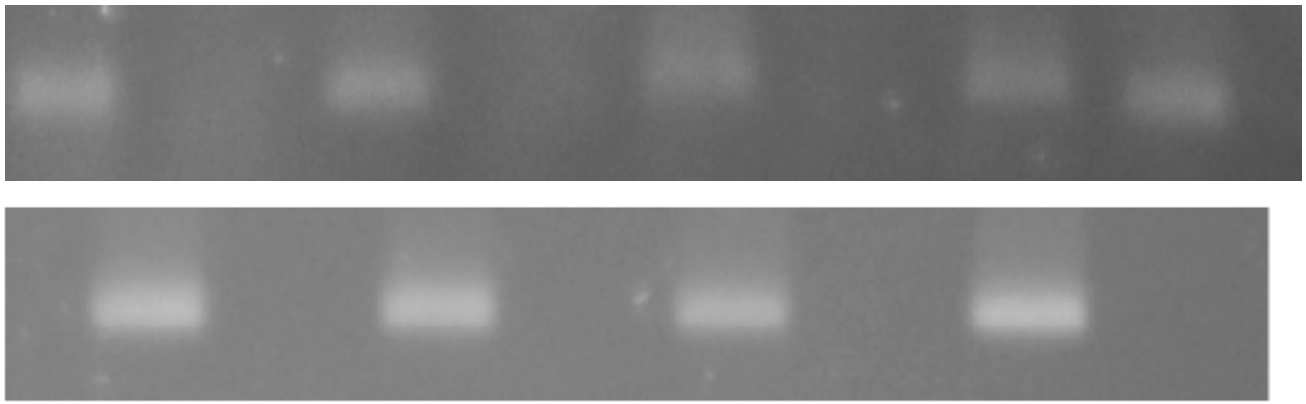
Dirs


LTR
Dirs ORF1
(350 BP)
LTR
ORF
1
ORF
2
ORF
3

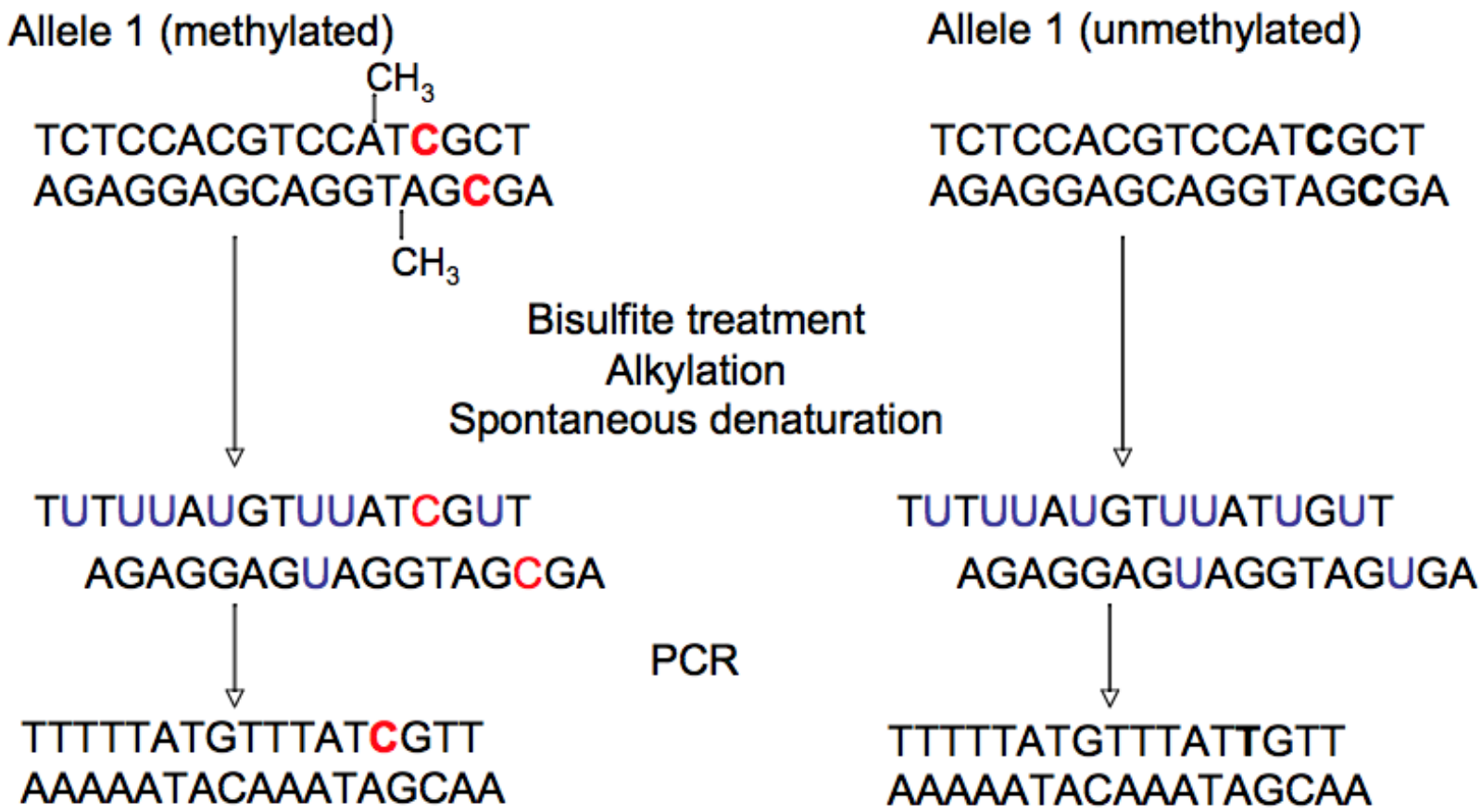

3dpf WT
RT+ RT-
3dpf mut
RT+ RT-
6dpf WT
RT+ RT-
6dpf mut
RT+ RT-
Dirs ORF1
Actin
Dirs1a

Dirs1a

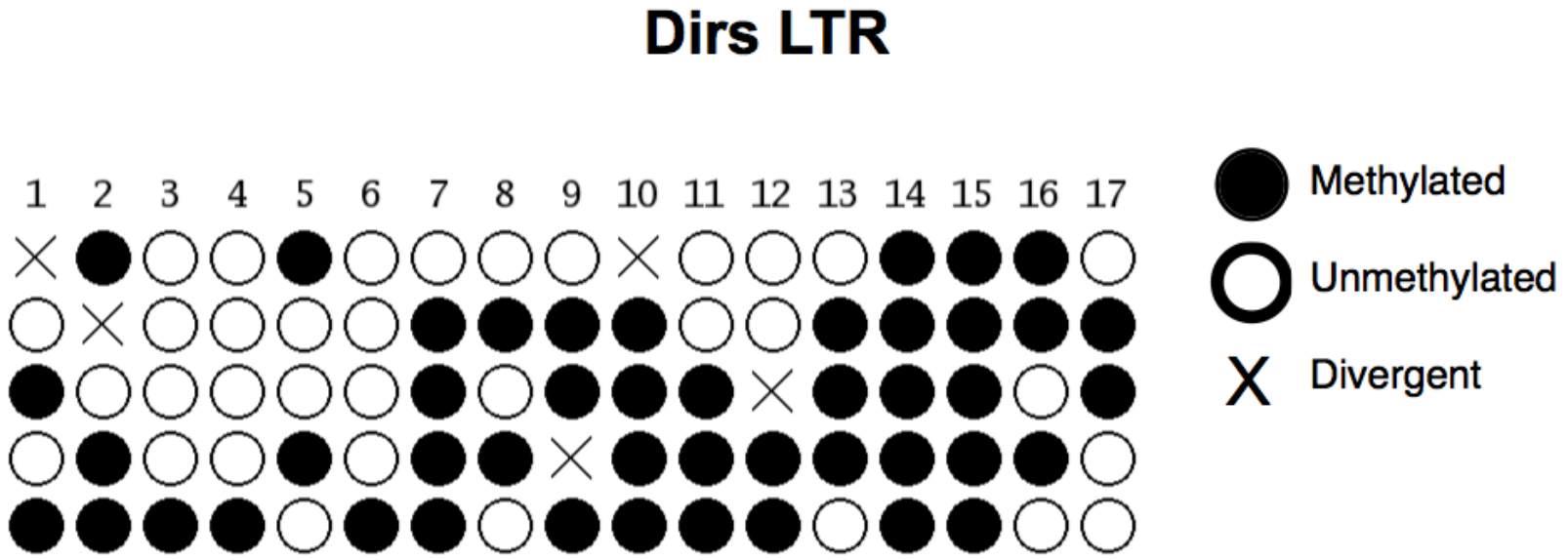
- Subfamily of Dirs1_DR retrotransposons
- 11% divergence between the DIRS1a_DR and DIRS1_DR consensus sequences.
- DIRS1a_DR has the presence of stop codons in ORF1-ORF3, while DIRS1_DR does not, suggesting that Dirs1 is non-autonomous



Detection of methylated cytosines with bisulfite conversion



Bisulfite conversion with Dirs LTR



Methylation of transposons is variable in WT genomes

52% methylated

Preliminary results suggest that transposons are not regulated by DNA methylation in zebrafish larvae.

Future Directions

- Examine expression of additional transposons in the zebrafish genome
- Investigate the levels of methylation in both WT and *dnmt1* mutant fish at transposon promoters
- Examine the role of *de novo* methyltransferases (Dnmt3's) in transposon control